Renewable and Sustainable Energy Reviews 14 (2010) 2596–2610

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Commercialization potential of microalgae for biofuels production

Jasvinder Singh [a,b,*], Sai Gu [a]

[a] Energy Technology Research Group, School of Engineering Sciences, University of Southampton, Southampton SO17 1BJ, UK
[b] Indian Institute of Petroleum, Mohkampur, Dehradun 248005, India

ARTICLE INFO

Article history:
Received 13 May 2010
Accepted 15 June 2010

Keywords:
Microalgae
Biofuel
Biorefinery
Biodiesel
Bioethanol
Biomethane

ABSTRACT

Microalgae feedstocks are gaining interest in the present day energy scenario due to their fast growth potential coupled with relatively high lipid, carbohydrate and nutrients contents. All of these properties render them an excellent source for biofuels such as biodiesel, bioethanol and biomethane; as well as a number of other valuable pharmaceutical and nutraceutical products. The present review is a critical appraisal of the commercialization potential of microalgae biofuels. The available literature on various aspects of microalgae, e.g. its cultivation, life cycle assessment, and conceptualization of an algal biorefinery, has been scanned and a critical analysis has been presented. A critical evaluation of the available information suggests that the economic viability of the process in terms of minimizing the operational and maintenance cost along with maximization of oil-rich microalgae production is the key factor, for successful commercialization of microalgae-based fuels.

Contents

1. Introduction . . . 2596
2. Why Algae?–advantages over first and second generation biomass feedstocks . . . 2597
   2.1. Life cycle assessment of biodiesel production from microalgae . . . 2597
   2.2. Variety and availability of algal biomass . . . 2598
3. Algae growth drives around the world . . . 2599
4. Algal biorefineries—scope and possibilities . . . 2601
   4.1. Concept of algal biorefinery . . . 2602
   4.2. Non-fuel products from microalgae . . . 2603
      4.2.1. Food supplements and fine chemicals of medicinal importance . . . 2603
      4.2.2. Livestock feed . . . 2604
   4.3. Biofuels products from algae—biodiesel, bioethanol and biomethane . . . 2604
      4.3.1. Biodiesel . . . 2604
      4.3.2. Bioethanol . . . 2605
      4.3.3. Biomethane . . . 2606
   4.4. Which way?—product- versus energy-based algal biorefinery . . . 2606
5. Future of algae-based biofuels . . . 2607
   5.1. Key strategies for successful algae biofuels commercialization . . . 2607
6. Conclusions . . . 2608
   References . . . 2608

1. Introduction

A constant rising worldwide demand of motor and power generation fuels, together with environmental concerns in terms of Green House Gases (GHG), has motivated the scientists and technologists to think about various alternate sources of energy. In recent years, a lot of thrust has been put on the search for the

* Corresponding author. Present address: Energy Technology Research Group, School of Engineering Sciences, University of Southampton, Southampton SO17 1BJ, UK. Tel.: +44 23 8059 8384/+91 135 2525784.
E-mail addresses: jas.singh@soton.ac.uk, jsingh@iip.res.in (J. Singh), s.gu@soton.ac.uk (S. Gu).

1364-0321/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rser.2010.06.014

potential biomass feedstocks from different sources, which can be converted to liquid as well as gas fuels for energy generation. Various biomasses have been identified as alternate source of energy fuels. This biomass ranges from various kinds of bio-wastes, e.g. food wastes, municipal wastes, agricultural wastes etc.; energy crops, e.g. edible as well as non-edible oilseeds; and various aquatic plants identified as bio-oil sources. In the recent years much thrust has been put on to examine the possibilities of using algae as a source of bio-oil and biogas for energy applications.

Algae are basically a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multi-cellular forms. These have the potential to produce considerably greater amounts of biomass and lipids per hectare than any kind of terrestrial biomass. As these can be cultivated on marginal lands, therefore do not compete with food or other crops. Algae can be cultivated photosynthetically using sunlight for energy and CO_2 as a carbon source. They may be grown in Shallow lagoons or raceway ponds on marginal land or closed ponds. Plastic tubes in ponds offer up to seven times the productivity of open ponds [1]. A number of closed photobioreactors are being investigated, for cost-effective production of the algae. These include horizontal tubes, vertical tubes, thin film and open/closed systems. Productivity is higher in the controlled, contained environment of a photobioreactor, but capitals as well as operating expenses are also substantially higher than for open systems.

The commercial viability of algae-based biofuels production shall eventually depend on economics of the technology. Regardless of whatsoever advances might come in terms of technological and biological innovations, the hard fact remains that the commercial marketplace shall have to have an enthusiasm for funding capital intensive energy projects, by ensuring the risk–return ratio to the acceptable stratum for debt and equity financiers.

The principal investment for an algae biomass project may be split into the costs associated with algal biomass growth, harvesting i.e. isolation of the biomass from the culture, dewatering or concentration of algae to a suitable level for further processing, and algal oil extraction systems. In addition, there are more traditional project costs such as engineering, permitting, infrastructure preparation, balance of plant, installation and integration, and contractor fees. Further to these, there are O&M costs which include expenses for nutrients (generally N-P-K), CO_2 distribution, and water replenishment due to evaporative losses, utilities, components replacement, and labour costs. In addition to capital and O&M costs, another significant expenditure is towards the costs of the land or leasing.

Significant speculation in research would be required to assure high levels of productivity that can match commercial-scale requirements. Worldwide a number of companies and government organizations have previously assessed different methodologies as well as designs and prepared cost estimates for commercial-scale production of algae. Many of these investigations recommend that algae to biofuels plants may be effectively developed on land adjacent to power stations, for converting the CO_2 from exhausts into fuel.

The present paper will provide a critical evaluation of the state-of-the-art along with techno-economic feasibility and commercialization potential of algae-based biofuels. A critical evaluation shall be presented of the current scenario of algae fuel production and implementation drive in various regions of the world including various parts of Asia, Europe and Americas.

2. Why Algae?–advantages over first and second generation biomass feedstocks

Algae were once considered to be 'aquatic plants' but are now classified separately because they lack true roots, stems, leaves, and embryos. While we refer to algae as feedstocks for biofuels, the definition includes all unicellular and simple multi-cellular microorganisms, including both prokaryotic microalgae, e.g. cyanobacteria (Chloroxybacteria), and eukaryotic microalgae, e.g. green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophta). The main advantages of microalgae-derived biofuels over the first and second generation biofuels are as follows.

Table 1
Typical oil yields from the various biomass sources in ascending order.

S.N.	Crop	Oil yield (l/ha)
1	Corn	172
2	Soybean	446
3	Peanut	1,059
4	Canola	1,190
5	Rapeseed	1,190
6	Jatropha	1,892
7	Karanj *(Pongamia pinnata)*	2,590
8	Coconut	2,689
9	Oil palm	5,950
10	Microalgae (70% oil by wt.)	136,900
11	Microalgae (30% oil by wt.)	58,700

Data sources: Chisti [3]; Lele [4]; http://journeytoforever.org/biodiesel_yield.html.

First of all, the microalgae can be produced all year round and therefore, quantity of oil production exceeds the yield of the best oilseed crops, e.g. biodiesel yield of 58,700 l ha^{-1} for microalgae containing only 30% oil by wt., compared with 1190 l ha^{-1} for rapeseed or Canola [2], 1892 l ha^{-1} for Jatropha [3], and 2590 l ha^{-1} for Karanj (*Pongamia pinnata*) [4]. Table 1 lists the typical oil yields from various sources. The rapid growth potential and numerous species of microalgae with oil content in the range of 20–50% dry weight of biomass is the another advantage for its choice as a potential biomass. The exponential growth rates can double their biomass in periods as short as 3.5 h [3–6]. Secondly, in spite of their growth in aqueous media, the algae need less water than terrestrial crops thus the load on freshwater sources is also reduced [7]. Due to this reason, the microalgae can also be cultivated in brackish water on non-arable land, and therefore may not incur land use change, minimizing associated environmental impacts [8], without compromising the production of food, fodder and other products derived from terrestrial crops [3]. According to Chisti [3], 1 kg of dry algal biomass utilizes about 1.83 kg of CO_2, thus the microalgae biomass production can help in bio-fixation of waste CO_2 with respect to air quality maintenance and improvement.

There is a dual potential for treatment of organic effluent from the agro-food industry for algae cultivation [9]. Apart from providing growth medium, the nutrients for its cultivation, e.g. nitrogen and phosphorus, can also be obtained from wastewater. A significant advantage to environment is that algae cultivation does not require herbicides or pesticides application [10]. In addition, these can also produce valuable co-products such as proteins and residual biomass after oil extraction, which may be used as feed or fertilizer [6], or fermented to produce bioethanol or biomethane [11]. The biochemical composition of the algal biomass can be mutated by varying growth conditions, and thus significantly boosting the oil yield [12]. Also the microalgae are capable of photo-biological production of 'bio-hydrogen' [13]. It therefore becomes rather imperative that the combination of potential biofuel production, CO_2 fixation, bio-hydrogen production, and bio-treatment of wastewater; as summarised above, accentuates the potential utilization of microalgae.

2.1. Life cycle assessment of biodiesel production from microalgae

In spite of a lot of thrust on third generation biofuel development, an adequate study of the life cycle assessment

(LCA) of biofuels' production from algae feedstocks is still not available. LCA studies reported by few authors lack in one aspect or other. Some of these studies present a comparative evaluation of the life cycle assessment of biofuel generation from various biomass feedstocks. Some have carried out LCA on the basis of lab-scale data after extrapolation. Some studies have not included all the factors required for an exact life cycle assessment. Most of these studies do not speak positive for the algae biofuels. This subsection presents the findings of the most recent studies carried out by some researchers, in this regard.

Lardon et al. [14] has carried out a life cycle assessment study based on an analysis of a hypothetical system, extrapolated from the lab-scale studies. They have used the inventory based on the figures derived from academic resources, communications with industrial producers, and inventories carried out on similar transformation units and processes described in the Ecoinvent database [15]. It lists certain standard rules for replacement and disposal of infrastructure, e.g. buildings, the concrete after its dismantling, steel-based and PVC products, electrical machinery etc. It is also mentioned by the authors [14] that for the assessments on electricity production with natural gas using industrial gas boilers, in which heat is produced, some rules based on the European energetic mix have been used. Further, for a process leading to the production of a number of products, an energy division has been done by segregating the environmental burden among co-products according to their relative energy contents. The authors have compared two different culture conditions i.e. nominal fertilizing or N_2 starving; two different extraction options i.e. dry or wet extraction and then the best alternative has been compared to first generation biodiesel and oil diesel. Their net energy debt analysis of the process chain reveals that only the wet extraction on low Nitrogen grown algae has a positive energy balance. The other scenarios lead to negative energy balance in spite of a 100% energy extraction from the oil cake. The outcome of their LCA confirms the potential of microalgae as an energy source but highlights the crucial inevitability of decreasing the energy and fertilizer consumption. Therefore control of nitrogen stress during the culture and optimization of wet extraction seem to be valuable options. Their study also highlights the potential of anaerobic digestion of oilcakes as an approach towards reducing the external energy demand and recycle of a part of the mineral fertilizers.

A recent LCA has also been reported by Clairens et al. [16]. The authors have compared the life cycle of algae feedstocks with three traditional terrestrial bio-crops namely corn, canola and switch grass. The authors have included typically all processes, e.g. climate requirements for cultivation of biomass, which include fertilizers, make-up water, global radiation isolation temperature and humidity requirements, and separation steps like precipitation and evaporation; material flow such as fertilizer requirements and energy requirements for pumping, mixing, tillage, sowing, chopping etc. The authors present a 'cradle to gate' analysis, which included all the products and process upstream of delivered dry biomass, rather than 'cradle to grave' approach which would include all processes till the oil production and utilization. The exclusion of the additional processing steps has been attributed to uncertainties surrounding the various processing steps, e.g. conversion of algae into liquid fuels [17], methods to produce liquid fuels from cellulosic feedstocks [18], and pay off from biofuel production versus bioelectricity production [19]. The biomass production model was based on several data sources including 30 years of metrological data [20,21]. The practicable unit for their analysis was chosen on the same order as primary energy consumption per annum of one American, two Japanese, or three polish citizens. The authors have also used life cycle inventory data from Ecoinvent database [15], as Lardon et al. [14]. The model has been run for the data taken at three locations in the United States of America. The algae production process has been modelled using open pond raceway configuration. The outcome of their analysis shows that energy production from the four crops is net positive, i.e. more energy is produced than consumed during biomass production. But terrestrial crops were found to have significantly lower energy use, as well as gas emission and water use than algae. These results imply that algae require more fossil-based carbon to produce the same amount of bioenergy. However, the land use is one impact on which algae offer a clear cut and appreciable improvement over corn, canola and switch grass. The results obtained for terrestrial crops are found to be in good harmony with the similar studies reported elsewhere [22].

A case study on comparison of life cycle analyses of microalgal biomass production has been reported by Jorquera et al. [23]. The authors have reported the net energy ratio (NER) for both the processes for microalgae production, e.g. tubular and flat bed reactors as well as raceway ponds. The NER of a system has been defined as the ratio of the total energy produced (energy content of the oil and residual biomass) over the energy content of photobioreactor construction and material plus the energy required for all plant operations:

$$\mathrm{NER} = \text{Net Energy Ratio} = \frac{\sum \text{Energy produced (lipid or biomass)}}{\sum \text{Energy requirements}}$$

The results indicate that the use of horizontal tubular photobioreactors (PBRs) is not economically feasible due to negative NER values. The NER values for flat bed PBRs and raceway ponds are found to be positive. The data for the life cycle assessment has been taken from Chisti [24] and the lipid content of the algae has been assumed to be 29.6% (dry wt. Lipid/dry wt. biomass). This value corresponds to *Nannochloropsis* species of the oil-rich microalgae. It is worth a mention here that the results obtained by the authors for flat bed bioreactors are contrary to the earlier reported results, where NER was reported to be negative by Huesemann and Benemann [25]. Also their results are more optimistic than those reported by Rodolfi et al. [26], where reported NER is not quite above 1. The setback of this study is that the authors did not consider the cost of microalgae harvesting and oil extraction.

The majority of the above reported life cycle analyses does not speak much positive about the advantage of microalgae-based oils over the oil obtained from terrestrial crops. However, none of these studies has been carried out using any commercial plant data. The sole reason seems to be non-availability of data from a suitable commercial plant as the commercial production of algae biofuels is yet in a budding state. All of these studies lack the data on the combustion of the bio-oils for energy generation. The studies conducted by Jorquera et al. also do not even consider the biomass harvesting data. The land use impact of microalgae offers a significant advantage over other bio-crops. Thus, it may be possible to utilize microalgae-based fuels cost-effectively, with a judicious processing. However, a detailed LCA carried out on larger scale plant may be further helpful.

2.2. Variety and availability of algal biomass

Consistent efforts are being made worldwide to achieve the ideal combination of algae species and growing conditions. The research is being done to cultivate the species with maximum lipid contents in order to make the microalgae conversion to biofuels more profitable as well as rewarding. Hundreds of species of microalgae are being experimented with, such as *Arthrospira platensis* (spirulina) which is easy to culture and easy to harvest but does not contain a high oil content and *Haematococcus pluvialis*

(red algae), which is very high in oil content. With the correct species in place and the right conditions, 1 ton of wet algae biomass can yield about 200 l of oil.

The three popular ways to grow microalgae for fuel are open pond system, closed pond systems, and engineered photobioreactors or PBR's. The closed pond systems are similar to open ponds but are covered for cooler climates. The open pond system requires constant 15 °C temperatures, less maintenance, is less expensive to set-up but does produce lower yields. Photobioreactors produce a higher yield per hectare, but have a higher start-up cost as well as require engineered maintenance. Recently, some companies in the US have been working towards bringing down the price of PBR's to almost the same cost as open ponds.

Significant hurdles are, however, yet to be overcome before microalgae to biofuel production becomes cost-effective and makes an impact to the world's supply of transport fuel. Some recent developments in technology have managed to surpass some of the problems making the process more energy efficient. The key issues need to be addressed are minimizing the capital and operational costs, cost of drying and extraction, and development work to increase productivity by developing more efficient harvesting systems. It is foreseen by the US industry that full commercialization of algae oil will begin to take place in the US in roughly 4–5 years.

3. Algae growth drives around the world

Piccolo [27] has reviewed some of the current developments for algae oil production in the EU countries. Some of the algae biofuel ventures in EU are as listed hereunder.

According to him the biggest algae investment in the EU is the £26 million publically funded project by the UK Carbon Trust which planned to build a large algae farm in Northern Africa. To support such development, Carbon trust launched a new £8 million research programme, Algae Biofuels Challenge (ABC) in 2009. The ABC has two phases [29]. Phase 1 is targeted on addressing the fundamental R&D challenges and phase 2 will focus on evolving the strategies for large-scale production of algae oil. The ABC is now led by a research team from 11 institutions including Universities of Manchester, New Castle, and Southampton, the Plymouth marine laboratory, and Scottish association for marine sciences [28]. Lately, the Scottish government launched an €6 million EU project called BioMara [30]. The scope of BioMara project is not just limited to the single celled algae species but also includes larger seaweed species which grow quickly and can be harvested for their biomass. In another development, a Spanish renewable energy company Aurantia and Green Fuel Tech of Massachusetts (USA) formed partnership through a $92 million project in 2007 to produce algae oil. In the long run, this project will target to scale up to 100 ha of algae greenhouses, producing 25,000 ton of algae biomass per annum. The plant will obtain its CO_2 from a cement plant near Jerez in Spain. In yet another endeavour, an Italian energy Company Eni, has installed a 1 ha pilot facility for algae oil production, in Gela, Sicily. This project is testing the photobioreactor facility as well as open ponds.

Piccolo feels that the countries with a coastline onto the Mediterranean Sea (roughly between 45 and 30° N), are suitable locations for algae farms, in particular in those countries south of the Mediterranean that experience warmer climates and whose temperature do not go too much below 15 °C throughout the year. This sort of warm climate of the Mediterranean region can facilitate the algae growth in the open or closed pond system. It would probably be the most efficient, economic and most suitable way to grow the algae biomass. New technologies in algae harvesting have also succeeded to set up cultivation for open pond farms to be located in slightly cooler climates by covering them with special material making them behave in a similar way as a greenhouse, this can certainly increase the latitude in which such farms can be built.

In view of the above, a number of countries in the Mediterranean basin possess a great potential for algae harvesting. Some countries, e.g. Israel, have been growing and harvesting algae for non-fuel i.e. medicinal purposes as well as nutrients production for a long time and also have begun the production of several strains for fuel production recently. The southern countries that border the Mediterranean Sea, e.g. Morocco, Algeria, Tunisia and Egypt, are particularly attractive because of high temperatures and enormous unused desert land. At the same time, countries like Libya, Cyprus and Turkey could also have lots of marginal land to harvest algae. Even if some of these countries do not have plenty of water resources that is also not a constraint. It is well established now that algae do not require freshwater, rather they can grow with recycled brackish or salty water. Besides, these countries are developing countries and could strongly benefit from such an industry. Algae farming can provide jobs for locals and the transfer of technologies to developing countries can also be beneficial for the country concerned.

A comprehensive survey has been carried out by Edward [31], which highlights the various aspects of the algal cultivation as well as utilization industry. In an appendix to his white paper, he has compiled the profiles of various companies around the world, which are engaged in the growth of algae for biofuel as well as other applications. He has classified the information on the basis of various algae farming technologies being used. The reported information has been summarised in Table 2. Fig. 1 shows a pie chart representing the percentage distribution of algal biofuel producing companies around the world in different regions. Fig. 2 represents the percentage distribution of various algae production technologies being used by the companies worldwide. Both of these figures are plotted on the basis of the data reported by Edward [31] as compiled in Table 2. These figures indicate that most of the companies contributing in development of algal biofuel drive are based in America. Some of these companies are however exploring the suitable regions for algae cultivation, but these are only few, using open pond systems or natural formations.

As indicated from Fig. 2, more thrust is being made to cultivate algae in closed systems or using photobioreactors. These bioreactors are in fact installed near a source of CO_2, and thus serve an additional purpose of carbon sequestration. However, the natural formations are the least capital cost intensive. The open ponds are also an attractive option for the regions where sufficient land can be allocated for algae growing, without interfering with the food chain.

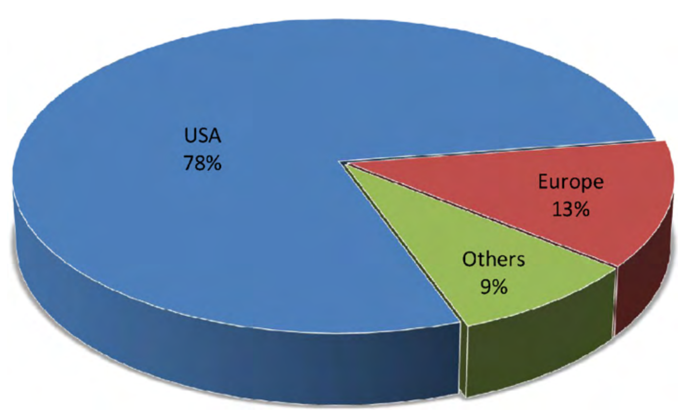


Fig. 1. Region-wise percentage of companies around the world producing algae fuels.

Table 2
Algae production companies around the world listed region-wise and course of action for biofuel conversion, listed on the various cultivation methods.

S.N.	Company	Region	Strategies/comments
Cultivation method: open ponds			
1	LiveFuels, Menlo Park California	USA	Instead of attempting to convert algae directly into biodiesel or ethanol, it is planned to create green crude, which may be used directly through the nation's current refinery system. They have created a national alliance of scientists led by a US Dept. of energy national lab that is focussing on producing bio-crude-oil by the year 2010.
2	OriginOil Inc., Los-Angeles, California	USA	Developing a technology to transform algae into true competitor to petroleum. The company claims that its patented technology will produce 'new oil' from algae, through a cost-effective and high-speed manufacturing process. This supply of new oil can be used for many products such as diesel, gasoline, jet fuels, plastics and solvents without the global warming effects
3	PetroSun, Scottsdale, Arizona	USA	Started the algae to biofuel production factory in Rio Honda, Texas in April 2007. The algae farm is a network of 1100 acres of saltwater ponds that the company thinks will make 4.4 million gallons of algal oil and 110 million pounds of biomass per year. The company also intends to extract algal oil on site at the farm and transport it to company biodiesel refineries. The company also plans to open more farms in Alabama, Arizona, Louisiana, Mexico, Brazil and Australia. Also created an algae-to-jet fuel team relationship with Science Applications International.
4	Neste Oil, Helsinki	European	Refining the imported vegetable oils and algae to make 170,000 ton of biodiesel per annum. The renewable fuel is suitable for all diesel engines and is strategic cornerstones for Neste. Its renewable fuel goal is to have 70% of its raw materials coming from non-food feedstocks in 10 years. By 2020 they want to have all raw materials coming from outside the food chain.
5	Ingrepo, Netherlands	European	A large biotech company specializes in industrial-scale algae production, plans to build algae production facilities at Malasia in collaboration with BioMac Sdn Bhd. Malasia was chosen due to its good weather conditions, infrastructure and government's interest in agro-biotechnology.
6	Seambiotic, Ashkelon Israel	Mediterranean	Founded in 2003, produces algae for a variety of applications including health foods, fine chemicals and biofuels. Working with the Israel Electric Company (IEC) using IEC's smokestack for a source of CO_2
Cultivation method: natural settings			
7	Kelco, San Diego	USA	The company harvests natural kelp beds with specially designed mowing machine. Transported by barges to the processing facility to produce alginic acid.
8	Neptune Industries, Boca Raton	USA	The company creates sustainable, eco friendly aquaculture with integrated solutions. In addition to hydroponically grown vegetables, lettuce, herbs and fish ponds, the company's patented Aqua-Sphere system uses fish waste to create additional revenue streams through the growth of algae for biofuels and methane gas.
9	Blue Marble Energy, Seattle	USA	The company utilizes hybridized bacteria to generate biochemical and bioenergy products. Their technology harnesses nutrients and converts polluted environments into natural biofactories for generation of renewable energy feedstock while cleaning the environment. They search for unwanted algae growth and have developed methods for cleaning polluted water where excess of nutrients leads to algal blooms. With their smart business model, the company gets paid to clean water and produce biomass that can be processed and sold. By addressing wild algae growth versus the traditional monoculture growth for biomass generation, the company keeps capital cost low and is able to produce a volume output that is multiples of closed systems or pond-based systems.
10	Aquaflow Binomics, New Zealand	New Zealand	A three-year old company, sources its algae from algae infested polluted water systems; cleaning the polluted environment in the process. Targeting to become the first company in the world to produce biofuel from wild algae harvested from open air environments. Harvests algae directly from settling ponds of standard effluent management systems and other nutrient-rich water. Expects to be able to provide a viable biofuel on a commercial scale.
11	Biofuel Systems, Spain	European	Developing a system for producing energy from marine algae, with the hope to replace the fossil fuels to reduce the pollution. Expects to produce huge amount of biopetroleum (their term for biodiesel) from phytoplankton in a limited space and moderate cost.
Cultivation method: closed systems			
12	A2BE Carbon Capture, Boulder Colorado	USA	The company builds carbon capture and recycle, CCR, systems that take advantage of Algae's capacity to profitably recycle industrial CO_2. An advanced combined energy conversion system combines algal CO_2 capture technology with biomass gasification and thus creates an integrated renewable fuel production system. A2BE offers a novel bio-harvesting technology where brine shrimp feed on the algae and the shrimp are harvested and processed. The patented system design Carbon Capture and Recycle (CCR) machine is climate adaptive due to thermal barriers above and below the culture flow that regulates temperature. This allows deployment nearly anywhere in sunshine.
13	GreenFuel Technologies Cambridge, Massachusetts	USA	Builds algal biofactory systems which use recycled CO_2 to feed the algae. The developed system can capture up to 80% of the CO_2 emitted from a power plant during the day when sunlight is available.

Table 2 (*Continued*)

S.N.	Company	Region	Strategies/comments
			The company claims that using its patented technology for growth on a 1 acre site, the amount of algae produced can be separated to the components which include 7000 gallon of jet fuel, 5000 gallon of ethanol, 1000 tons of protein for foods, 200 pounds of specialized nutrients, and 20 pounds of pigments.
14	Solazyme, Inc. San Francisco	USA	A five-year old biotechnology company that harnesses the power of microalgae to produce clean and scalable high-performance oil, biofuels and "green" chemicals. The company ignores the sun and grows algae in dark in large tanks where they are fed sugar to supercharge their growth. It is claimed that it is a 1000 times more productive than natural processes. Using its technology to make speciality oils for cosmetic industry. Demonstrated that their algal diesel "Solodiesel" has superior cold weather climate suitability, where conventional biodiesel is not usable.
15	Algeneol Biofuels, Fort Meyers, Florida	USA	The company founded in 2006 to develop industrial-scale algaculture systems to make ethanol from algae on desert land using seawater and CO_2. Patented a technology with blue–green algae, cyanobacteria that are N_2 fixing which reduces their fertilizer costs. Plans to make ethanol with blue–green algae that produce oil and then secrete it. The fuel can be taken directly from the algal tanks while the algae continue to thrive. This process uses significantly less energy than drying and pressing the biomass for oil. Plans to make 100 million gallon of ethanol annually in Mexico's Sonaran Desert by the end of 2009, which shall be increased to 1 billion gallons by 2012.
16	Sapphire Energy, San Diego	USA	Company launched in May 2007, and initiated a new biofuel category called green crude production. The company has built a revolutionary molecular platform that converts sunlight and CO_2 to renewable, carbon neutral alternatives to conventional fossil fuel without the downsides of current biofuel efforts. Products are chemically identical to molecules in the crude oil, making company's products entirely compatible with the current energy infrastructure—cars, refineries, and pipelines. Company will not reveal the type of algae they use but it is most likely a genetically modified cyanobacteria, blue–green algae. The advantage of this form of algae is that the algae secrete the bio-crude oil which rises to the top and can be skimmed. This saves on harvesting and processing costs.
17	Inventure Chemical technology, Seattle	USA	Company working on a patent pending algae-to-jet fuel product and has produced algae-based fuel in 10 gallon tests. The algae used for biofuel production is sourced from facilities in Israel, Arizona, and Australia.
18	Solena, Washington State	USA	Company uses its patented plasma technology to gasify algae and other organic substances with high energy outputs. The company is talking with Kanas power firm Sunflower to build a 40 MW power plant which will run on syn-gas produced from gasified algae. The algae would be grown in big plastic containers and fed sunlight and sodium bicarbonate, which is by-product of an adjacent coal plant.
19	Solix Biofuels, Fort Collins, Colorado	USA	Company founded in April 2006, intends to use microalgae to create a commercially viable biofuel that will play a vital role in solving climate change and petroleum scarcity without competing the global food supply. Proposed to build its first large-scale facility at the nearby New Belgian Brewery, where CO_2 produced during beer production would be used to feed the algae.
20	XL Renewables, Phoenix, Arizona	USA	Company changed its name from XL Dairy Group and started a patent pending algal production system called Simgae for simple algae, in 2007. It uses common agriculture and irrigation components to produce algae at a fraction of the cost of competing systems. The water used is fortified to enhance the production and pumped back to the troughs to a harvest system where algae are extracted. Water is then recycled back. CO_2 is injected periodically.
21	Aurora Biofuels	USA	The company uses the genetically modified algae to efficiently create biodiesel using a patented technology, developed at University of California, Berklay. The company claims to create biofuel with yields 125 times higher and have 50% less costs than current production methods.
22	Bionavitas, Snoqualmie, Washington	USA	Company claims a patent pending process for high volume production of algae using biofactories. The competitive advantage claimed is to be a "lighting system" that includes one or more light emitting substrates configured to light at least some of a plurality of photosynthetic organism retained in the bioreactor.
23	Cellena, Hawaii	USA	A joint venture created by algae-to-biofuel start-up HR Biopetroleum and Shell oil. The company had announced a new process for extracting algae oil without using chemicals drying or an oil press.

4. Algal biorefineries—scope and possibilities

The biorefinery concept is akin to today's petroleum refineries, which produce multiple fuels and products (petrochemicals) from petroleum. The various petrochemicals produced from the refineries find their applications in various industries, e.g. plasticizers, vegetable oil extraction, paints and anti-corrosive substances, and many more. The production of these petrochemicals makes the refinery process more profitable as well as practical. Industrial biorefineries have been identified as the most promising route to the creation of a new domestic bio-based industry. On the basis of correspondence with a petroleum refinery, a biorefinery may be defined as a facility that integrates biomass conversion processes and equipment to produce fuels,

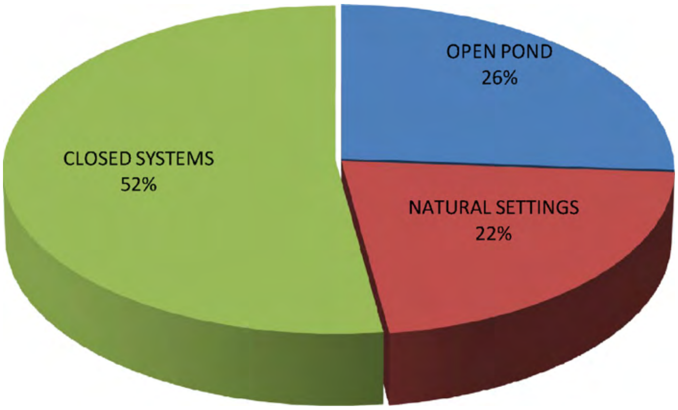


Fig. 2. Worldwide technologies being used for algae biofuel production companies.

Table 3
Proteins and carbohydrates contents from various species of microalgae.

S.N.	Algae strain	Protein (% dwt)	Carbohydrate (% dwt)
1	*Scenedesmus obliquus*	50–56	10–17
2	*Scenedesmus quadricauda*	47	–
3	*Scenedesmus dimorphus8*	8–18	21–52
4	*Chlamydomonas rheinhardii*	48	17
5	*Chlorella vulgaris*	51–58	12–17
6	*Chlorella pyrenoidosa*	57	26
7	*Spirogyra* sp	6–20	33–64
8	*Dunaliella bioculata*	49	4
9	*Dunaliella salina*	57	32
10	*Euglena gracilis*	39–61	14–18
11	*Prymnesium parvum*	28–45	25–33
12	*Tetraselmis maculate*	52	15
13	*Porphyridium cruentum*	28–45	40–57
14	*Spirulina platensis*	52	8–14
15	*Spirulina maxima*	28–39	13–16
16	*Synechoccus* sp.	46–63	15
17	*Anabaena cylindrical*	43–56	25–30

Data source: Becker [86].

power, materials and/or chemicals from biomass. A more specific and comprehensive definition of a biorefinery has been given by IEA Bioenergy Task 42 document [32], which states, "the sustainable processing of biomass into a spectrum of marketable products and energy". By producing multiple products, a biorefinery can take advantage of the differences in biomass components and intermediates and maximize the value derived from the biomass feedstock.

4.1. Concept of algal biorefinery

The concept of algal biorefinery has the key *raison d'être* to explore the possibility of producing biofuels at large scale from industrially grown algae. The main components of a typical algae feedstock are proteins, carbohydrates, lipids, and other valuable components, e.g. pigment, anti-oxidants, fatty acids, vitamins etc. Fig. 3 shows a flowchart listing main components of microalgae [33]. The protein and carbohydrate contents in various strains of microalgae are high, up to 50% of its dry weight. Table 3 lists the protein and carbohydrate contents of some strains of microalgae. The maximum lipid contents in microalgae are also around 40% on wt. basis, which is reasonably good. All these factors make microalgae a potential source for bio-oil production. Table 4 shows lipid contents for some algae strains identified for biofuel extraction.

A wide array of products can be formed from the algae [34,35]. These products range from the food supplements and nutrients for human, livestock feed, fine organic chemicals for pharmaceuticals,

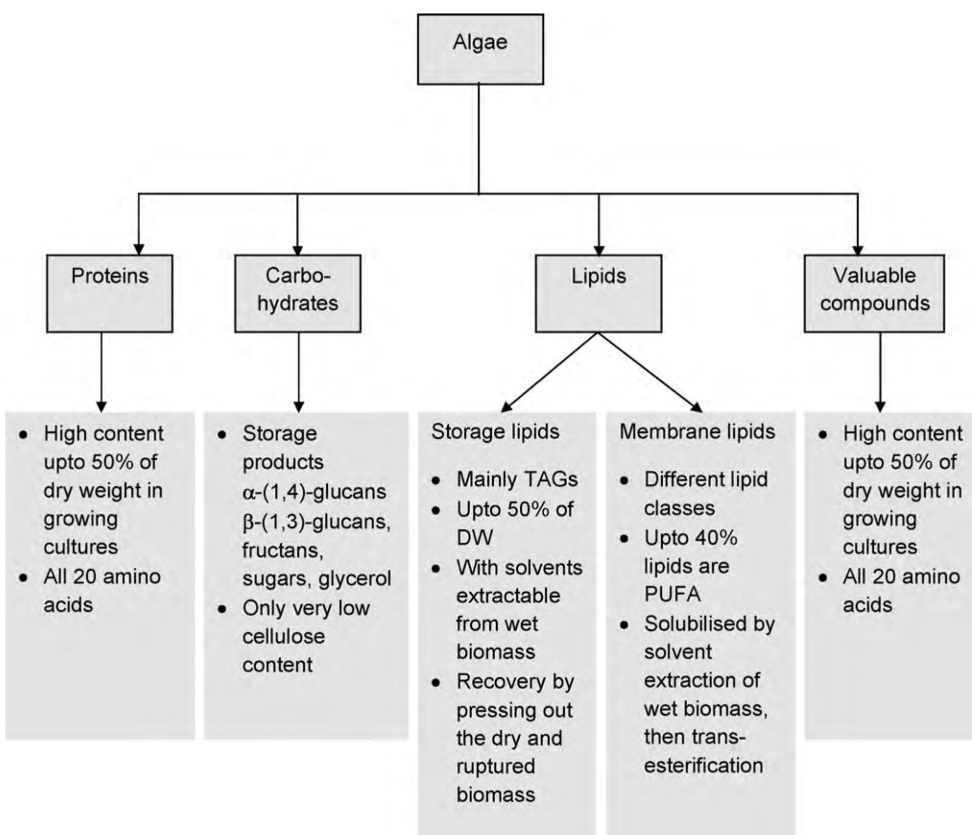


Fig. 3. Components of typical microalgae (33).

Table 4
Lipid contents and lipid productivity of the 30 microalgal strains cultivated in 250 ml flasks (Rodolfi et al. [10]).

Algal group	Microalgae strains	Habitat	Lipid content (% biomass)	Lipid productivity (mg/l/d)
Diatoms	*Chaetoceros muelleri F&M-M43*	Marine	33.6	21.8
	Chaetoceros calcitrans CS 178	Marine	39.8	17.6
	P. tricornutum F&M-M 40	Marine	18.7	44.8
	Skeletonoma costatum CS 181	Marine	21	17.4
	Skeletonoma sp. CS 252	Marine	31.8	27.3
	Thalassioria pseudonana CS 173	Marine	20.6	17.4
	Chlorella sp. F&M-M48	Freshwater	18.7	42.1
	Chlorella sorokiniana IAM-212	Freshwater	19.3	44.7
	Chlorella vulgaris CCAP 211/11b	Freshwater	19.2	32.6
	C. vulgaris F&M-M49	Freshwater	18.4	36.9
Green algae	*Chlorococcum sp. UMACC 112*	Freshwater	19.3	53.7
	Scenedemus quadricauda	Freshwater	18.4	35.1
	Scenedemus F&M-M19	Freshwater	19.6	40.8
	Scenedemus sp. DM	Freshwater	21.1	53.9
	T. suecica F&M-M33	Marine	8.5	27
	Tetraselmis sp. F&M-M34	Marine	14.7	43.4
	T. suecica F&M-M35	Marine	12.9	36.4
	Ellipsoidion sp. F&M-M31	Marine	27.4	47.3
	Monodus subterraneus UTEX 151	Freshwater	16.1	30.4
	Nannochloropsis sp. *CS 246*	Marine	29.2	49.7
Eustigmatophytes	*Nannochloropsis* sp. *F&M-M26*	Marine	29.6	61
	Nannochloropsis sp. *F&M-M27*	Marine	24.4	48.2
	Nannochloropsis sp. *F&M-M24*	Marine	30.9	54.8
	Nannochloropsis sp. *F&M-M29*	Marine	21.6	37.6
	Nannochloropsis sp. *F&M-M28*	Marine	35.7	60.9
	Isochrysis sp. (T-ISO) CS 177	Marine	22.4	37.7
	Isochrysis sp. F&M-M37	Marine	27.4	37.8
Prymnesiophytes	*Pavlova salina CS 49*	Marine	30.9	49.4
	Pavlova lutheri CS 182	Marine	35.5	50.2
Red algae	*Porphyridium cruentum*	Marine	9.5	34.8

Data source: Rodolfi et al. [10]; Khan et al. [65].

pigments and various other applications, e.g. chlorophyll, biobutanol and acetone etc., along with energy fuels, e.g. biodiesel, bioethanol, and biomethane. Following subsections present a brief account of the various valuable products that can be obtained from various algae feedstocks.

4.2. Non-fuel products from microalgae

4.2.1. Food supplements and fine chemicals of medicinal importance

Microalgae are a potential source of various food supplements and biomaterials used in pharmaceutical industry. Some of these are omega-3 fatty acids, eicosapentanoic acid (EPA), decosahexaenoic acid (DHA) and chlorophyll. Omega-3 fatty acids are generally obtained from fish oil. But inadequate supplies of fish oil in recent years, coupled with problems arising due to its unpleasant taste and poor oxidative stability, have rendered this route less promising [36]. It is indicated in the literature [34] that microalgae naturally contain omega-3 fatty acid which can be purified to provide a high-value food supplement. The practical sources of omega-3 in microalgae are normally eicosapentanoic acid (EPA) and decosahexaenoic acid (DHA). In comparison to fish, microalgae are self-producing omega-3, thus make the process straightforward as well as economical [37].

Apart from the production of omega-3, EPA as well as DHA has its individual pharmaceutical applications. EPA finds its medicinal use treatment of heart and inflammatory diseases: asthma, arthritis, migraine headache and psoriasis [38]. A number of research papers have published on the bulk microalgae culture aimed at EPA production. Hu et al. have reported the cultivation of marine microalga, Pavlova viridis in 60 l outdoor photobioreactor and presented a comparison with indoor cultivation, in their research paper [39]. They observed lower total fatty acid, but higher yield of EPA compounds from outdoor photobioreactor system as compared to indoor system. Hence, they suggest outdoor system for EPA production as more suitable method in comparison with the indoor one [39]. Besides this study, Cheng-Wu et al. [40] have also reported the use of outdoor photobioreactor to cultivate *Nannochloropsis* sp. and produced EPA. They have also reported the seasonal variations on EPA yields. Their investigations indicate up to 35% higher yield in summer as compared to that in winter. Chini Zittelli et al. [41] also found photobioreactors' more advantages in controlling contaminants, as compared to open pond system, while studying the culture of *Nannochloropsis* sp. In their investigations, temperature and irradiance of photobioreactors was not found to have any significant affect on the EPA yields [41]. In spite of all these investigation discussed above, a cost-effective system for culturing microalgae is essential to be developed in order to meet demands of EPA.

The clinical uses of DHA include prevention and cure of cancer, AIDS, heart disease, to control and lower cholesterol, boost immune system, and body detoxification. Amount of DHA produced have significantly been affected by types of microalgae. It has been reported by Patil et al. [42] that marine microalgae containing significantly more DHA contents as compared to fresh water microalgae; mainly consist saturated or monounsaturated fatty acids. Jiang et al. [43] have reported total fatty acid contents in a range of 33–39% in *Schizochytrium mangrove* marine microalgae, having main component of DHA. It has also been verified by Vazhappilly and Chen [44] that *Crypthecodinium cohnii* had DHA content up to 19.9% of total fatty acid against other microalgae species studied, e.g. 17.0% in *Amphidium caryerea* and 16.1% in *Thrautocytrium aureum*. Patil et al. also found *Isochrysis galbana* to have significant amount of DHA with the specific productivity around 0.16 g/l/d [42]. In addition, a study by Carvalho and Malcata [45] indicated that amount of CO_2, light intensity, operation mode (batch and continuous) significantly affect productivity of DHA.

Approximately 1.29 mg/l/d of DHA was obtained under optimized conditions.

Chlorophyll is another pharmaceutically important product obtained from the microalgae. Almost all algae cultured under optimum condition are believed to contain around 4% of overall cell weight of chlorophyll on dry weight basis [34]. It has been reported by some researchers that cyanobacteria, popularly known as blue–green algae, typically contain chlorophyll-a, whereas other species of green algae mostly have chlorophyll-b [46,47]. Chlorella has been reported to have high amount of chlorophyll among various species of microalgae [48]. A chelating agent activity of chlorophyll renders it suitable to be used in ointment, treatment for pharmaceutical benefits especially liver recovery and ulcer treatment. Further to that, it repairs cells, increases haemoglobin in blood and boosts the cell growth [49]. Chlorophyll has also been investigated as source of pigments in cosmetics. The brown and red algae are mostly used in the cosmetics industries [50]. Besides, in food industry, chlorophyll is used as natural pigment ingredient in processed foods [51]. Because of its strong green pigment and consumers' demand for natural foods, chlorophyll is gaining importance as food additive. This in turn is encouraging food processors to switch from artificial pigments to chlorophyll-based natural colouring for better public health. Nevertheless, a downstream process needs to be developed to isolate chlorophyll-a and -b from algae.

4.2.2. Livestock feed

The livestock feed is another useful product which may be obtained from the algae. Many of algae have been examined by various researchers, for their biochemical compositions for their suitability as a substitute or primary livestock feed. It has been reported that microalgae also play a key role in high-grade animal nutrition food, from aquaculture to farm animals. Comprehensive nutritional and toxicological evaluations have demonstrated suitability of algae biomass as a valuable feed supplement or substitute for conventional animal feed sources [52]. Dhargalkar and Verlecar [52] have reported in their investigations that certain edible seaweeds can be used as food due to lower calorie, high concentration of minerals, vitamins and proteins and a low fat content. Spirulina, a well-known blue–green alga, is still used in food supplements due to its excellent nutrient compounds and digestibility [53]. In addition to high protein content of 60–70 wt.%, Spirulina is also believed to be a rich source of vitamins, particularly vitamin B12 and β-carotene and minerals [54]. The cultivation of Spirulina is possible in high saline water and alkaline conditions, in comparison to other microorganisms. This property imparts it an advantage to serve as a feedstock for livestock feed. Besides, Red algae, mainly Porphyra and brown algae, particularly Laminaria, Undaria, and Hizikia fusiforme, were identified as safe for human consumption as food [55]. Another species Chlorella has been suggested as such potential food, principally because it consists of complete nutrients required for human nourishment [56].

Many studies have reported the use of algae as aquaculture feed. Microalgae species *Hypnea cervicornis* and *Cryptonemia crenulata* particularly rich in protein were tested in shrimp diets [57]. The studies carried out by da Silva and Barbosa [57] reveal that the amount of algae in fish meal results in significant increase in shrimp growth rates. Besides, better growth weight and protein efficiencies ratio was observed by Azaza et al. [58] in case of Tilapia fish farming with algae as nutritional food source in feed [58]. Also, Phorphidium valderianum, marine cyanobacteria were successfully used as feed for aquaculture based on their nutritional and non-toxic performance [54].

In addition to its importance in aquaculture, Spolaore et al. [56] have reported that up to 5–10% of conventional protein sources in poultry feed can effectively be replaced by algae. Ginzberg et al. [59] studied role of algae, *Porphyridium* sp. as feed supplement on metabolism of chicken. Their investigations show that cholesterol of egg yolk was reduced about 10% and colour of egg yolk became darker, indicating higher content of carotenoid. Belay et al. [60] reviewed potential of Arthrospira (Spirulina) in animal feed. Although Arthrospira is widely used as food additive and can replace 50% of protein diets in existing feeds, it was concluded that protein sources from soya and fish meal were more cost-effective and thus preferred to Arthrospira [51]. Furthermore, a study on the addition of *Laminaria digitata* suggested that algae supplemented feed increased pig weight up to 10% on a daily basis [61].

4.3. Biofuels products from algae—biodiesel, bioethanol and biomethane

4.3.1. Biodiesel

Biodiesel is the monoalkyl ester of long-chain fatty acids derived from renewable feedstocks, such as vegetable oil or animal fats [62]. The primary advantages of biodiesel are that it is one of the most renewable fuels and also non-toxic and biodegradable [63]. Presently, biodiesel has come to mean a very specific chemical modification of natural oils. Oilseed crops such as rapeseed and soybean oil have been extensively evaluated as sources of biodiesel by various authors [62–65]. One of the biggest advantages of biodiesel compared to many other alternative transportation fuels is that it can be used in existing diesel engines without modification, and its suitability for blending in at any ratio with petroleum diesel.

Any biofuel production process, which can successfully replace an equivalent conventional fuel, needs to fulfil three basic requirements. First, a sufficient feedstock to produce fuel at commercial scale should be produced, secondly it should cost less than conventional fossil fuel, and it should match standard specification of fuel quality. The oilseed crops mentioned above as well as other identified non-food crops, e.g. Jatropha curcas, switch grass and Karanj (*Pongamia pinnata*) etc., are known to interfere with the land and food chain crops up to some extent.

The viability of microalgae for biodiesel production has been studied by a number of authors. A comprehensive review on it has been presented by Teressa Mata et al. [64]. Khan et al. [65] has also presented a critical evaluation on prospects of biodiesel production microalgae. They have emphasized the need to explore the possibilities of producing biodiesel from microalgae, as it will not compete with the land and cereal crops. Microalgae are potential to be used as a raw material for biodiesel production, as it meets all of these requirements. They possess high growth rate and provide lipids fraction for biodiesel production [66]. Microalgal lipids are mostly neutral lipids with lower degree of unsaturation. This makes microalgal lipids a potential replacement for fossil fuel. Teressa Mata et al. [64] have presented a comprehensive review of the microalgae for biodiesel production. He points out that, in spite of dependence of oil yield on the particular algal strain, the oil contents of microalgae are generally much higher than the other vegetable crops. Table 5 lists the oil contents of some typical microalgae feedstocks in use. The data has been compiled from various sources from literature [3].

Various methods for extraction of lipids from microalgae have been reported in literature, but most common methods are expeller/oil press, liquid–liquid extraction (solvent extraction), supercritical fluid extraction (SFE) and ultrasound techniques. Table 6 lists the advantages and limitations of the popular extraction methods. Oil presses or expellers are the most common method for extraction of oil from nuts and seeds [67]. Same equipment and process would be appropriate for extraction of oil from microalgae. To ensure the efficacy of this process, algae must

Table 5
Oil contents of microalgae.

Microalgae	Oil content (% dwt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis suecica	15–23
B. braunii	25–75

Adapted from: Chisti [3].

first need to be dried. Press uses pressure to break cells and compress out oil. Though this method extracts almost 75% of oil and no special skill is required, this conventional method has been reported to be less effective due to relatively longer extraction time [67].

Another method for lipid extraction from microalgae is solvent extraction. In this approach, algae paste in water is extracted by adding organic solvents, such as benzene, cyclo-hexane, hexane, acetone, chloroform etc. Solvent destroy algal cell wall, and extract oil from aqueous medium because of their higher solubility in organic solvents than water. The oil may be separated from the solvent extract by distillation process. Latter can be recycled for further use. Hexane is reported to be the most efficient solvent in extraction based on its highest extraction capability and low cost [74,75]. Besides, Fajardo et al. [76] have reported an improved lipid extraction method by using a two-step process. In first step ethanol is used for extraction of the lipids and then in second step, hexane is used for purifying the extracted lipids. They have reported about 80% of lipid recovery yields by this two-stage extraction. Butanol has also been shown effective in extracting lysophospholipids. But the shortfall of this method is that butanol is high boiling solvent and thus difficult to evaporate and secondly it tends to extract more impurities due to its high polarity [77]. A study by Morrison and Conventry [78] reported that fatty acids were nearly always more extractable at 100 °C as compared to ambient temperature, particularly saturated acids, e.g. C16:0 palmitic and C18:0 stearic acid. However, polyunsaturated acids, e.g. C18:2 omega6 and C18:3 omega3 acids gave slightly lower yields with hot propanol–water (3:1, v/v), water saturated butanol, methanol and methanol–water (85:15, v/v). In another study reported by Pratoomyot et al. [79] found that fatty acid content in microalgae varied between different species when extracted using chloroform:methanol (2:1, v/v) as a solvent. However, this particular method is unsuitable due to the use of environmentally destructive solvents.

Another commonly employed method of extraction is supercritical fluid extraction (SFE) [70], which is extremely time efficient. It makes use of high pressures and temperatures to rupture the cells. The effect of temperature and pressure has been studied by Canela et al. [80]. Their studies reveal that the temperature and pressure of SFE did not have any effect on yield of extracted compounds. However, it has influenced the extraction rate. Andrich et al. [81] have investigated the kinetics of SFE in extraction of Nanochloropsis sp. to produce bioactive lipid (polyunsaturated fatty acids, PUFA). PUFA profile was about the same when different SFE conditions applied (temperature range between 45 and 55 °C and pressure 400–700 bar). However, SFE system and solvent extraction using hexane were found to give similar results on lipid extraction. Further studies by Andrich et al. [82] with *Spirulina platensis* for extraction of PUFA found that SFE system gave higher yield and fatty acid composition compared to the solvent extraction.

Another competent mechanism to be used in extraction of microalgae is via ultrasound. This method involves the exposure of algae to a high-intensity ultrasonic wave, which creates tiny cavitation bubbles around cells. Collapse of bubbles emits shockwaves, shattering cell wall thus disrupting the later and releasing desired compounds into solution. Wiltshire et al. [83] have reported more than 90% extraction of fatty acids and pigments from Scenedesmus obliquus, using this method. Any sort of breakdown or alteration to products has not been reported to be observed during extraction process. Subsequently, Pernet and Tremblay [84] have reported their research work using ultrasound for complete extraction of lipids from *Chaetoceros gracilis*. Effect of storage time and treatment method on the yield of lipid extracts has been studied. It was concluded that ultrasonic increased extraction rate thus affected recovery of lipid extracts throughout the study. In spite of the extensive use of ultrasound technique for extraction of oil from microalgae at laboratory scale, sufficient information on feasibility or economics for a commercial-scale operation is still not well reported in literature. Despite a lot of apparent potential in this extraction approach, further research is needed to be done for commercial applications.

4.3.2. Bioethanol

In general, two methods are normally adopted for production of bioethanol from biomass. The first one is biochemical process, i.e. fermentation and other is by thermo-chemical process or gasification. Most of the biomass feedstocks used for generation of bioethanol, such as corn and sugarcane, have same difficulty as biomass for biodiesel production. The primary problem being the high-value for food applications and requires large quantities of land to be produced for both kinds of feedstocks. Thus, both of these compete with food chain as well as land use, which pose a constraint to expand production of these biofuels [85].

The recent attempts for producing ethanol are focusing on microalgae as a feedstock for fermentation process. Microalgae are rich in carbohydrates and proteins that can be used as carbon

Table 6
Advantages and limitations of various extraction methods for algae oil (Harun et al. [34]).

Extraction methods	Advantages	Limitations	References
Oil press	Easy to use, no solvent involved	Large amount of sample required, slow process	[64]
Solvent extraction	Solvent used are relatively inexpensive; reproducible	Most organic solvents are highly flammable and/or toxic; solvent recovery is expensive and energy intensive; large volume of solvent needed	[68,69]
Supercritical fluid extraction	Non-toxicity (absence of organic solvent in residue or extracts), 'green solvent' used; non-flammable, and simple in operation	Often fails in quantitative extraction of polar analytes from solid matrices, insufficient interaction between supercritical CO_2 and the samples	[70,71]
Ultrasound	Reduced extraction time; reduced solvent consumption; greater penetration of solvent into cellular materials; improves the release of cell contents into the bulk medium	High power consumption; difficult to scale up	[72,73]

sources for fermentation. Table 3 lists the amount of carbohydrates and protein measured from various algal species. Bacteria, yeast or fungi are microorganisms used to ferment carbohydrates to produce ethanol under anaerobic conditions. In addition to ethanol as main products, CO_2 and water are also formed as by-products. According to following simplified reaction equation below, stoichiometric yields are 0.51 kg ethanol and 0.49 kg CO_2, per kg of carbon sugar, i.e. glucose.

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2.$$

The prospective feedstocks and feasibility of the various processes for ethanol production have been reported by various authors. However, bioethanol production by fermentation has not been reported extensively. The most recent work on bioethanol production by fermentation has been reported by Harun et al. [87]. They have carried out experiments, for studying the suitability of microalgae (Chlorococum sp.) as a substrate, using yeast for fermentation. A productivity level of around 38 wt.% has been reported by the authors, which supports the suitability of microalgae as a promising substrate for bioethanol production. In his doctoral work, Moen [88] has demonstrated that brown seaweed produces higher bioethanol compared to other algae species. Another study by Hirayama et al. [89] proposed a self fermentation of algae to obtain ethanol. The reported advantages of this technique over conventional fermentation are a comparatively simple process with shorter fermentation time. Ueda et al. [90] have patented a two-stage process for microalgae fermentation. In the first stage, microalgae undergo fermentation in anaerobic and dark environment and ethanol is produced. Then the ethanol thus produced can be purified to be used as fuel. The CO_2 produced in the fermentation process was recycled to algae cultivation ponds as a nutrient to grow microalgae. The second stage involved the utilization of remaining algae biomass slurry left after fermentation, which may be used in anaerobic digestion process while keeping the pH in the range of 6–9. This process produced methane which can further be converted to produce electricity [90]. Bush and Hall [91] pointed out certain shortfalls in the patented process of Ueda et al. and have patented a modified fermentation process by adding yeasts, *Saccharomyces cerevisiae* and *Saccharomyces uvarum*, to algae fermentation broth for ethanol production. According to Bush and Hall [91], the process patented by Ueda et al. [81] was not commercially scalable due to the inherent limitations of single cell free floating algae. A study by Hon-Nami [92] indicated that *Chlamydomonas perigranulata* was fermented to produce ethanol, butannediol, acetic acid and CO_2. They found that hydrogen recovery from that fermentation was about 139% and carbon recovery at around 105%.

Although limited reports on algae fermentation are available, a number of advantages have been reported in the production of bioethanol from algae. Fermentation process involves less intake of energy and the process is much simple in comparison of biodiesel production system. In addition, CO_2 produced as by-product from fermentation process can be recycled as carbon sources for microalgae cultivation, thus reducing the greenhouse gases emissions as well. However, the technology for the commercial production of bioethanol from microalgae is yet under development stage and is being further investigated.

4.3.3. Biomethane

As evident from some of the patented processes mentioned above [90,91], it is imperative that the bioethanol production by fermentation is also useful for simultaneous production of biogas. This reason has resulted in considerable attention towards the application of methane fermentation technology to algae to produce valuable by-products such as biogas. Biogas produced from anaerobic microorganisms by anaerobic digestion mainly consists of a mixture of methane (55–75%) and CO_2 (25–45%). Methane from anaerobic digestion can be used as fuel gas and also be converted to generate electricity [93]. Residual biomass from anaerobic digestion also can further be reprocessed to make fertilizers. In addition to being renewable and sustainable, this would encourage sustainable agricultural practices in providing greater efficiencies and reduce algae production costs. Due to absence of lignin and lower cellulose, microalgae exhibit good process stability and high conversion efficiency for anaerobic digestion [94]. Table 7 shows methane yield from different algae species as feedstock. The biogas production from this anaerobic digestion process is primarily affected by its organic loadings, pH, temperatures, and retention time in reactors. Mainly long solid retention time and high organic loading rate give significant results in high methane yield [95]. In addition, anaerobic digestion can operate in either mesophilic (35 °C) or thermophilic (55 °C) conditions.

Table 7
Methane yield from the different algae strains.

Biomass	Methane yield ($m^3\,kg^{-1}$)	Reference
Laminaria sp.	0.26–0.28	[95]
Gracilaria sp.	0.28–0.4	[96]
Macrocystis	0.39–0.41	[95]
L. Digitata	0.5	[97]
Ulva sp.	0.2	[98]

Otsuka and Yoshino [99] used constant temperature, mesophilic for anaerobic digestion of Ulva sp. and found 180 ml/g (volatile solid based) of methane yield. On the other hand, Golueke et al. [100] reported that mesophilic condition promoted slower breakdown of organic compounds in anaerobic digestion process. However, it was reported that production cost of methane from microalgae was higher compared to other biomass, grass and wood [101]. The integrated processes that combine algae cultivation and wastewater treatment system for methane production can be most suitable approach to reduce production cost and make it more profitable. The earliest mention of the use of wastewater ponds to cultivate algae and harvesting algal sludge for production of biogas by anaerobic digestion to produce biogas has been made by Oswald and Gotaas [102]. Since then, a few works have been done to study in detail about this system [103]. It can be concluded that system could avoid eutrophication process and improve pond waste nutrient treatment [104]. Although microalgae offer a good potential for biogas production, commercial productions have still not been implemented.

4.4. Which way?—product- versus energy-based algal biorefinery

It is quite evident from the discussions in the above subsections that microalgae are a potential source for a number of very useful products, apart from biofuel production. The algae are also being used for generation of other products, described above, for a long time. It is also evident from the data compiled in Table 2, many companies around the world are trying to produce 'green crude oil' or bio-crude or bio-oil, which may be converted to various fuels by various conversion processes [31]. Now the question arises what should be the ideal configuration of an algal biorefinery? Shall we go for a product-based or energy-oriented biorefinery?

But at the same time a hybrid refinery may be a more profitable venture, rather than exclusively product-based or energy-based biorefinery. Bennett points out that as feedstock, algae could fit into most of the integrated biorefinery designs that are on the drawing board as its primary components might be optimized to produce more oils, carbohydrates or proteins [105]. The challenge

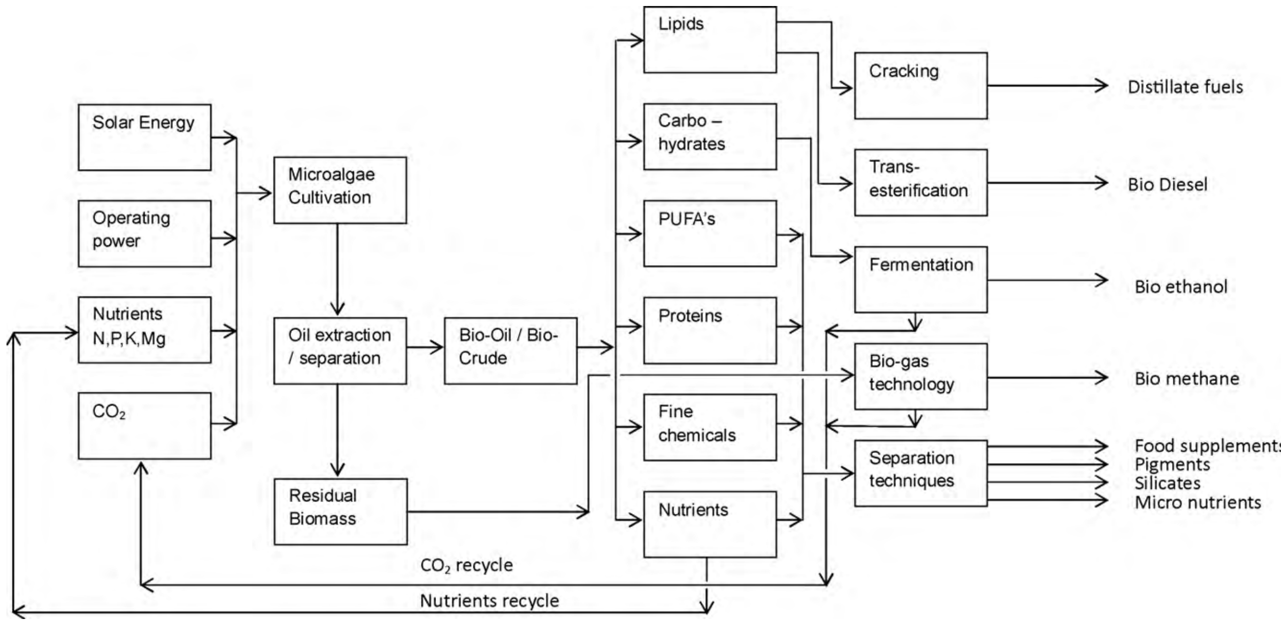


Fig. 4. Proposed schematic flow sheet for a microalgae biorefinery.

lies in the identification of better species and reducing the costs of production, which are currently an order of magnitude above other biofuel systems. As with other biorefinery ideas, the use of algae presents numerous routes to the future integration of raw materials, processes and products to create a hybrid biorefinery. Proposed schematic flow sheets for hybrid algae bio-refinery is presented in Fig. 4.

5. Future of algae-based biofuels

Algae biofuels have now generated plenty of interest with over 150 companies worldwide [106,107]. As evident from Table 2, numbers of large and mid-size American as well as European companies are launching their algae biofuel projects. This drive includes some major companies, e.g. ExxonMobil Corp and BP Amoco plc. ExxonMobil Corp has allocated $600 M over the next 5–6 years for its partnership with Synthetic Genomics. BP Amoco plc has earmarked $10 M for a project with Martek Biosciences Corp. Also Dow Chemical Co. and Algenol Biofuels Inc. have announced a joint pilot project aimed at making ethanol from algae-based biorefinery. The US Department of Energy has already published the draft of their 'National Algal Biofuels Technology Roadmap' and the final version of the document is expected soon. According to the document [108], the future of algae biofuels is quite bright in many ways. Some market analysts feel that in spite of all the positive factors, e.g. fast growth potential, non-interference with food crops, CO_2 sequestration etc., the successful commercial implementation of algal biofuel shall depend on the development of high-value co-products, e.g. renewable polymers or pigments. In view of this the pathway to algae biofuel commercialization is witnessing several breakthroughs and technological advances in synthetic biology, metabolic engineering and genomics; development of closed loop bioreactor systems and raceway ponds; harvesting, lighting and extraction systems.

5.1. Key strategies for successful algae biofuels commercialization

A recent study Algae 2020 [109] has identified five key strategies for a successful algae biofuels commercialization drive. In a series of articles published in various issues of *Biofuel international* magazine, Thurmond has highlighted various strategic steps required for successful algae biofuel commercialization [110–112]. These strategies are fatter, faster, cheaper, easier and fractionation marketing approaches to help producers to reduce costs and accelerate the commercialization of algae biodiesel, bio-crude, and drop-in fuels [111]. The first two i.e. 'fatter' and 'faster' are the primary strategic needs for algae fuel commercialization drive. The primary need for the algae biofuels producers is to identify algae species that have a high oil content that will also grow quickly to produce biodiesel, bio-crude and drop-in fuels. Algae producers are especially interested in using algal species with a high triglyceride (TAG) oil content for biodiesel and bio-crude production. Compared to most algae used today with 25% oil content, several scientists and producers are working on identifying species and methods to increase oil content. Most algae systems today can generate from 2500 gallon up to 5000 gallon of oil per surface acre in raceway ponds acre using 30% oil content.

It is largely agreed among seasoned practitioners, phycologists, producers, and subject matter experts that some algae varieties with high oil content such as *Botryococcus braunii* (Bb) grow slowly and can be harvested only a few times a week, whereas algae with lower oil content such as Duniella or Nannocloropsis (in the 20–40% range) will grow more quickly and can be harvested daily or a few times a day. If algae producers can use fatter algae with 60% oil content, they can reduce the size and footprint of algae biofuels production systems by as much as half, resulting in significant capital and operating costs reductions and savings for systems twice their size using species with lower oil content. This presents a significant innovation and a welcome improvement for algae producers eager to lower costs to enter biofuels markets.

The commercialization of algae biofuels is also dependent on the economics of the process. At the same time ease of implementation is another important factor for success of a new technology or process. In view of this the 'cheaper' and 'easier' process are the next important strategies. On the basis of several available reports, the Algae 2020 study has reported the estimated costs to produce algae oils and algae biodiesel today between $9 and $25 per gallon in ponds, and $15–$40 in photobioreactors (PBRs). Since algae production systems are a complex composite of several sub-sets of systems (i.e. production, harvesting, extraction, drying systems), reducing the number of steps in algae biofuels production is essential to providing easier, better, and lower cost systems.

A crucial economic challenge for algae producers is to discover low cost oil extraction and harvesting methods. With the advent of cheaper photobioreactors (PBRs), these costs are likely to come down significantly in the next few years. In the present scenario, reducing these costs is critical to algae biofuel companies for its successful commercial implementation. Extraction systems with estimates up to $15 per gallon of oil produced depending on the extraction method can be less than cost-effective. For example, Origin Oil has developed a technology to combine harvesting and extraction systems into a single process that is designed to reduce system complexity and costs for algae producers. Another example is to employ a method that uses algae cells as mini-processors and refineries in a process referred to as 'milking the algae' that will consume CO_2 and excrete hydrocarbon fuels directly.

One company, Algae to Energy, uses a patented system from Missing Link Technology that can extract algae oil from 0.08 up to $0.29 per gallon (depending on the species used) compared to other algae extraction methods ranging from $2 a gallon up to $12 per gallon.

Another example is a harvesting technology from Algae Venture Systems that costs less than $0.30 per gallon of oil harvested compared to traditional centrifuge technologies which can cost up to $1 or more per gallon. Cost reductions in algae production systems are essential for algae producers to establish economically sustainable and profitable enterprises.

Examples of this include Arizona State's blue–green algae that excrete a kerosene type of jet fuel and Algenol's blue–green algae that excrete ethanol fuel directly. There are also a few species of algae that will naturally excrete oils from the cells. By milking the algae, these algal micro-refineries help to bypass the harvesting, extraction and refining systems all together by excreting forms of biofuels directly from the cells. These methods have the capability to significantly reduce production costs, and help to simplify complex processes for emerging algae producers and customers of new algae biofuels production systems.

Finally the co-production of some more valuable fraction and their marketing is also important for the success. Even with algae species with up to 50% oil content, the additional 50% of the biomass remains. This biomass fraction contains valuable proteins for livestock, poultry and fish feed additives valued from $800 up to $2500 per tonne, as described above in Section 4.2.

Other fractions of the algae contain valuable chemicals or molecular compounds that can be used to produce green plastics, green detergents, cleaners, and polymers that are bio-degradable, non-toxic, and can be sold at a premium price over traditional petroleum-based products. These biomass co-product marketing strategies will be critical to the success of aspiring algae biodiesel, bio-crude, renewable diesel, aviation fuel and drop-in fuel producers.

Therefore, it may be concluded that a hybrid biofuel refinery concept can be implemented profitably for microalgae-based biofuels. CO_2 and nutrients may be recycled for microalgae culture, and thus help in carbon sequestration. The thermal conversion as well as chemical conversion processes may be successfully used for fuel generation. Gasification process may be integrated with power generation. Apart from fuels, e.g. biodiesel, bioethanol and biomethane, other valuable products can be co-generated to make the commercialization process a profitable venture. Thus, the future of algae biofuel looks bright. Of course certain quantum of research is still needed, which is being done by the people around the world.

6. Conclusions

A critical assessment of the commercialization potential of algae biofuels has been presented. The suitability of algae feedstocks for conversion to biofuels has been explored. It seems that new technologies, e.g. tubular PBRs shall enhance the production of microalgae feedstocks for various fuel productions along with CO_2 recycle for algae culture and thus reduce the pollution.

Some life cycle analyses have been presented by few authors. A critical analysis of these studies reveals that an adequate LCA study is still not available which may help to present a clear picture of the situation. The reason is non-availability of commercial plant data. It may be hoped that with more and more companies coming forward in microalgae conversion business, a more detailed picture will emerge out. Since the microalgae feedstocks are non-competing with land use change as well as food crops, the scope of their implementation looks great.

It is obvious from the critical appraisal of the viability of algae projects from a true market perspective that total fixed costs along with recurring costs will be a decision-making step to the future commercialization of the algae-based biofuels. More innovations are still needed for the development of technologies which reduce costs while increasing the yields. This can be realized successfully through a coherent, extensive, and well-funded R&D program. It is extremely important in the early phases of this promising, yet challenging industry, to deliberate new business models that look at the bioenergy potential of algae through the transportation fuels market, as well as production of other higher value products so as to make the economics practicable. A sustained effort from the technologists and planners can result in the successful accomplishment of this extremely potential concept towards the solution of world's future energy concerns.

References

[1] Algal biofuels–Algae for production of biofuels, http://www.biofuelstp.eu/algae.html.

[2] Schenk P, Thomas-Hall S, Stephens E, Marx U, Mussgnug J, Posten C, et al. Second generation biofuels: high-efficiency microalgae for biodiesel production. BioEnergy Res 2008;1(1):20–43.

[3] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25(3):294–306.

[4] Lele S. Indian Green Energy Awareness Center, http://www.svlele.com/karanj.htm.

[5] Metting FB. Biodiversity and application of microalgae. J Ind Microbiol 1996;17(5–6):477–89.

[6] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J Biosci Bioeng 2006;101(2):87–96.

[7] Dismukes GC, Carrieri D, Bennette N, Ananyev GM, Posewitz MC. Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Curr Opin Biotechnol 2008;19(3):235–40.

[8] Searchinger T, Heimlich R, Houghton RA, Dong F, Elobeid A, Fabiosa J, et al. Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. Science 2008;319(5867):1238–40.

[9] Cantrell KB, Ducey T, Ro KS, Hunt PG. Livestock waste-to-bioenergy generation opportunities. Bioresour Technol 2008;99(17):7941–53.

[10] Rodolfi L, Zittelli GC, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 2008;102(1):100–12.

[11] Hirano A, Ueda R, Hirayama S, Ogushi Y. CO_2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation. Energy 1997;22(2–3):137–42.

[12] Qin J. Bio-hydrocarbons from algae—impacts of temperature, light and salinity on algae growth. Barton, Australia: Rural Industries Research and Development Corporation; 2005.

[13] Ghirardi ML, Zhang L, Lee JW, Flynn T, Seibert M, Greenbaum E, et al. Microalgae: a green source of renewable H2. Trends Biotechnol 2000; 18(12):506–11.

[14] Lardon L, Helias A, Sialve B, Steyer Jean–Philippe, Bernard O. Life cycle assessment of biodiesel production from microalgae. Environ Sci Technol 2009;43(17):6475–81.

[15] Frischknecht R., Althaus H.-J., Doka G., Dones R., Heck T., Hellweg S.,et al. Overview and methodology. Final report ecoinvent v2.0 No. 1, Swiss Centre for Life Cycle Inventories; 2007.

[16] Clairens AF, Resurreccion EP, White Mark A, Colos Lisa M. Environmental life cycle comparison of algae to other bioenergy feedstocks. Environ Sci Technol 2009;44(5):1813–9.

[17] Yang YF, Feng CP, Inamori Y, Maekawa T. Analysis of energy conversion characteristics in liquefaction of algae. Resour Conserv Recycl 2004; 43(1):21–33.
[18] Miao X, Wu Q, Yang C. Fast pyrolysis of microalgae to produce renewable fuels. J Anal Appl Pyrol 2004;71(2):855–63.
[19] Campbell JE, Lobell DB, Field CB. Greater transportation energy and GHG offsets from bioelectricity than ethanol. Science 2009;324(5930):1055–7.
[20] NOAA, Surface Radiation Network (SURFRAD), National Oceanic and Atmospheric Administration, Boulder, CO, 2009.
[21] NREL, National Solar Radiation Database (1961–1990), National Renewable Energy Laboratory: Golden, CO, 1994.
[22] Kim S, Dale BE. Cumulative energy and global warming impact from the production of biomass for biobased products. J Ind Ecol 2004;7(3–4):147–62.
[23] Jorquera O, Kiperstok A, Sales Emerson A, Embiruçu M, Ghirardi Maria L. Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. Renew Bioresour Technol 2010;101: 1406–13.
[24] Chisti Y. Biodiesel from microalgae beats bioethanol. Trends Biotechnol 2008;26(3):126–31.
[25] Huesemann M, Benemann JR. Biofuels from microalgae: review of products, process and potential, with special focus on Dunaliella sp. In: Ben- Amotz A, Polle JEW, Subba Rao VD, editors. The alga Dunaliella: biodiversity, physiology, genomics and biotechnology. New Hampshire: Science Publishers; 2009.
[26] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 2009;102:100–12.
[27] Piccolo A. Algae oil production and its potential in the Mediterranean region 1st EMUNI Research Souk 2009 (EMUNI ReS 2009). The Euro-Mediterranean Student Research Multi-conference Unity and Diversity of Euro-Mediterranean Identities, 9 June 2009.
[28] www.businessgreen.com/business-green/news/2259794/carbon-trust-launches-algae.
[29] http://www.carbontrust.co.uk/News/presscentre/2008/algae-biofuels-challenge.htm.
[30] http://www.biomara.org/.
[31] Edward M. The algal industry survey—a white paper by Dr. Mark Edward & Centre for management technology; 2009.
[32] Biorefineries: adding value to the sustainable utilization of biomass. IEA Bioenergy: T42:2009:01.
[33] Schmid-Straiger U. Algae biorefinery–Concept. National German Workshop on Biorefineries, 15 September 2009, Worms.
[34] Harun R, Singh M, Forde Gareth M, Danquah Michael K. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew Sust Ener Rev 2010;14:1037–47.
[35] Brennan L, Owende P. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Ener Rev 2010;14:557–77.
[36] Luiten EEM, Akkerman I, Koulman A, Kamermans P, Reith H, Barbosa MJ, Sipkema D, et al. Realizing the promises of marine biotechnology. Biomol Eng 2003;20:429–39.
[37] Belarbi EH, Molina E, Chisti Y. A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. Process Biochem 2000;35:951–69.
[38] Singh S, Kate BN, Banecjee UC. Bioactive compounds from cyanobacteria and microalgae: an overview. Critical Rev Biotechnol 2005;25:73–95.
[39] Hu C, Li M, Li J, Zhu Q, Liu Z. Variation of lipid and fatty acid compositions of the marine microalga Pavlova viridis (Prymnesiophyceae) under laboratory and outdoor culture conditions. World J Microbiol Biotechnol 2008;24: 1209–14.
[40] Cheng-Wu Z, Zmora O, Kopel R, Richmond A. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). Aquaculture 2001;195:35–49.
[41] Chini Zittelli G, Lavista F, Bastianini A, Rodolfi L, Vincenzini M, Tredici MR. Production of eicosapentaenoic acid by *Nannochloropsis* sp. cultures in outdoor tubular photobioreactors. J Biotechnol 1999;70:299–312.
[42] Patil V, Kallqvist T, Olsen E, Vogt G, Gislerod HR. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquaculture Int 2007;15: 1–9.
[43] Jiang Y, Fan KW, Wong RTY, Chen F. Fatty acid composition and squalene content of the marine microalga *Schizochytrium mangrove*. J Agric Food Chem 2004;52:1196–200.
[44] Vazhappilly R, Chen F. Eicosapentaenoic acid and docosahexaenoic acid production potential of microalgae and their heterotrophic growth. J Am Oil Chem Soc 1998;75:393–7.
[45] Carvalho AP, Malcata FX. Optimization of omega-3 fatty acid production by microalgae: crossover effects of CO_2 and light intensity under batch and continuous cultivation modes. Marine Biotechnol 2005;7:381–8.
[46] Deng Z, Hu Q, Lu F, Liu G, Hu Z. Colony development and physiological characterization of the edible blue–green alga, Nostoc sphaeroides (Nostocaceae, Cyanophyta). Prog Nat Sci 2008;18:475–1484.
[47] Dring JM. The biology of marine plants, 2nd ed., Cambridge University Press; 1991. p. 46.
[48] Nakanishi K. Chlorophyll rich and salt resistant chlorella. European Patent 1,142,985; 2001.
[49] Puotinen CJ. Herbs for detoxification. McGraw-Hill Professional; 1999. p. 25.
[50] Erica MT. SalonOvations' day spa techniques. Cengage Learning; 1996, 137.
[51] Humphrey AM. Chlorophyll as a colour and functional ingredient. J Food Sci 2004;69:422–5.
[52] Dhargalkar VK, Verlecar XN. Southern Ocean seaweeds: a resource for exploration in food and drugs. Aquaculture 2009;287:229–42.
[53] Kumar M, Sharma MK, Kumar A. Spirulina fusiformis: a food supplement against mercury induced hepatic toxicity. J Health Sci 2005;51:424–30.
[54] Thajuddin N, Subramanian G. Cyanobacterial biodiversity and potential applications in biotechnology. Curr Sci 2005;89:47–57.
[55] Besada V, Andrade JM, Schultze F, Gonzalez JJ. Heavy metals in edible seaweeds commercialised for human consumption. J Mar Sys 2009;75: 305–13.
[56] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J Biosci Bioeng 2006;101:87–96.
[57] da Silva RL, Barbosa JM. Seaweed meal as a protein source for the white shrimp Litopenaeus vannamei. J Appl Phycol 2008;1–5.
[58] Azaza MS, Mensi F, Ksouri J, Dhraief MN, Brini B, Abdelmouleh A, et al. Growth of Nile tilapia (Oreochromis niloticus L.) fed with diets containing graded levels of green algae ulva meal (Ulva rigida) reared in geothermal waters of southern Tunisia. J Appl Ichthyol 2008;24:202–7.
[59] Ginzberg A, Cohen M, Sod-Moriah UA, Shany S, Rosenshtrauch A, Arad S. Chickens fed with biomass of the red microalga *Porphyridium* sp. have reduced blood cholesterol level and modified fatty acid composition in egg yolk. J Appl Phycol 2000;12:325–30.
[60] Belay A, Kato T, Ota Y, Spirulina. (Arthrospira): potential application as an animal feed supplement. J Appl Phycol 1996;8:303–11.
[61] He ML, Hollwich W, Rambeck WA. Supplementation of algae to the diet of pigs: a new possibility to improve the iodine content in the meat. J Animal Physiol Animal Nutrition 2002;86:97–104.
[62] Meher LC, Vidya SD, Naik SN. Technical aspects of biodiesel production by transesterification—a review. Renew Sust Energy Rev 2006;10: 248–68.
[63] Van Gerpen J. Biodiesel processing and production. Fuel Process Technol 2005;86:1097–107.
[64] Mata Teresa M, Martins Antonio A, Caetano Nidia S. Microalgae for biodiesel production and other applications: a review. Renew Sust Energy Rev 2010;14:217–32.
[65] Khan SA, Rashmi, Hussain Mir Z, Prasad S, Banerjee UC. Prospects of biodiesel production from microalgae in India. Renew Sust Energy Rev 2009;13:2361–72.
[66] Song D, Fu J, Shi D. Exploitation of oil-bearing microalgae for biodiesel. Chin J Biotechnol 2008;24:341–8.
[67] Popoola TOS, Yangomodou OD. Extraction, properties and utilization potentials of cassava seed oil. Biotechnology 2006;5:38–41.
[68] Herrero M, Ibanez E, Senorans J, Cifuentes A. Pressurized liquid extracts from *Spirulina platensis* microalga: determination of their antioxidant activity and preliminary analysis by micellar electrokinetic chromatography. J Chromatogr A 2004;1047:195–203.
[69] Galloway JA, Koester KJ, Paasch BJ, Macosko CW. Effect of sample size on solvent extraction for detecting cocontinuity in polymer blends. Polymer 2004;45:423–8.
[70] Macias-Sanchez MD, Mantell C, Rodriguez M, Martinez De La Ossa E, Lubian LM, Montero O. Supercritical fluid extraction of carotenoids and chlorophyll a from Nannochloropsis gaditana. J Food Eng 2005;66:245–51.
[71] Pawliszyn J. Kinetic model of supercritical fluid extraction. J Chromatogr Sci 1993;31:31–7.
[72] Luque-Garcia JL, Luque De Castro MD. Ultrasound: a powerful tool for leaching. TrAC Trends Anal Chem 2003;22:41–7.
[73] Martin PD. Sonochemistry in industry. Progress and prospects. Chem Ind (London) 1993;7:233–6.
[74] Demirbas A. Production of biodiesel from algae oils. Energy Sources Part A—Recovery Utilization Environ Eff 2009;31:163–8.
[75] Serrato AG. Extraction of oil from soybeans. J Am Oil Chem Soc 1981;58: 157–9.
[76] Fajardo AR, Cerdan LE, Medina AR, Fernandez FGA, Moreno PAG, Grima EM. Lipid extraction from the microalga Phaeodactylum tricornutum. Eur J Lipid Sci Technol 2007;109:120–6.
[77] Xiou JY, Xu Y. Analysis of lysophospholipids in human body fluids: comparison of the lysophospolipid content in malignant vs non-malignant disease. In: Feng Li, Glen D, editors. Functional lipidomics. CRC Press; 2005. p. 125.
[78] Morrison WR, Conventry AM. Solvent extraction of fatty acids from amylase inclusion complexes. Starch 1989;41:24–7.
[79] Pratoomyot J, Srivilas P, Noiraksar T. Fatty acids composition of 10 microalgal species. J Sci Technol 2005;27:1179–87.
[80] Canela APRF, Rosa PTV, Marques MOM, Meireles MAA. Supercritical fluid extraction of fatty acids and carotenoids from the microalgae Spirulina maxima. Ind Eng Chem Res 2002;41:3012–8.
[81] Andrich G, Nesti U, Venturi F, Zinnai A, Fiorentini R. Supercritical fluid extraction of bioactive lipids from the microalga *Nannochloropsis* sp.. Eur J Lipid Sci Technol 2005;107:381–6.
[82] Andrich G, Zinnai A, Nesti U, Venturi F, Fiorentini R. Supercritical fluid extraction of oil from microalga Spirulina (Arthrospira) platensis. Acta Alimentaria 2006;35:195–203.
[83] Wiltshire KH, Boersma M, Moller A, Buhtz H. Extraction of pigments and fatty acids from the green alga Scenedesmus obliquus (Chlorophyceae). Aquat Ecol 2000;34:119–26.

[84] Pernet F, Tremblay R. Effect of ultrasonication and grinding on the determination of lipid class content of microalgae harvested on filters. Lipids 2003;38:1191–5.
[85] Sun Y, Cheng J. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 2002;83:1–11.
[86] Becker EW. Oil production. In: Baddiley, et al., editors. Microalgae: biotechnology and microbiology. Cambridge University Press; 1994.
[87] Harun R, Danquah MK, Forde Gareth M. Microalgal biomass as a fermentation feedstock for bioethanol production. J Chem Technol Biotechnol 2010; 85:199–203.
[88] Moen E. Biological degradation of brown seaweeds. Doctoral thesis submitted to Norwegian University of Science and technology (1997). Referred by Kelly M S and Dworjanyn S., in "The potential of marine biomass for anaerobic biogas production" Argyll, Scotland: Scottish Association for Marine Science Oban; 2008.
[89] Hirayama S, Ueda R, Ogushi Y, Hirano A, Samejima Y, Hon-Nami K, et al. Ethanol production from carbon dioxide by fermentative microalgae. Stud Surf Sci Catal 1998;114:657–60.
[90] Ueda R, Hirayama S, Sugata K, Nakayama H. Process for the production of ethanol from microalgae. U.S. Patent 5,578,472; 1996.
[91] Bush RA, Hall KM. Process for the production of ethanol from algae. U.S. Patent 7,135,308; 2006.
[92] Hon-Nami K. A unique feature of hydrogen recovery in endogenous starchto-alcohol fermentation of the marine microalga, *Chlamydomonas perigranulata*. Appl Biochem Biotechnol 2006;131:808–28.
[93] Holm-Nielsen JB, Al Seadi T, Oleskowicz-Popiel P. The future of anaerobic digestion and biogas utilization. Bioresour Technol 2009;100:5478–84.
[94] Vergara-Fernandez A, Vargas G, Alarcon N, Velasco A. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. Biomass Bioenergy 2008;32:338–44.
[95] Chynoweth DP. Renewable biomethane from land and ocean energy crops and organic wastes. HortScience 2005;40:283–6.
[96] Bird KT, Chynoweth DP, Jerger DE. Effects of marine algal proximate composition on methane yields. J Appl Phycol 1990;2:207–13.
[97] Morand P, Briand X. Anaerobic digestion of Ulva sp. 2. Study of Ulva degradation and methanisation of liquefaction juices. J Appl Phycol 1999;11:165–77.
[98] Briand X, Morand P. Anaerobic digestion of Ulva sp. 1. Relationship between Ulva composition and methanisation. J Appl Phycol 1997;9: 511–24.
[99] Otsuka K, Yoshino A. A fundamental study on anaerobic digestion of sea lettuce Ocean'04–MTS/IEEE Techno-Ocean'04: bridges across the oceans–conference proceedings; 2004: p. 1770–3.
[100] Golueke GC, Oswald JW, Gotaas BH. Anaerobic digestion of algae. Appl Environ Microbiol 1957;5:47–55.
[101] Chynoweth DP, Fannin FK, Srivastava JV. Biological gasification of marine microalgae. In: Bird KT, Benson PH, editors. Seaweed cultivation for renewable resources. Amsterdam: Elsevier; 1987. p. 287–303.
[102] Oswald JW, Gotaas HB. Photosynthesis in sewage treatment. Trans Am Soc Civ Eng 1957;122:73–105.
[103] Oswald WJ, Bailey Green F, Lundquist TJ. Performance of methane fermentation pits in advanced integrated wastewater pond systems. Water Sci Technol 1994;30:287–95.
[104] Brune DE, Collier JA, Schwedler TE, Eversole AG. Controlled eutrophication system and process. U.S. Patent 7,258,790; 2007.
[105] Simon Bennett. Algal biorefineries. Cleantech magazine, July 2008, http://www.cleantechinvestor.com/portal/biofuels/1758-algal-biorefinery.html.
[106] Biodiesel Magazine, Oct 2009 (http://www.biodieselmagazine.com). Algal biofuels create excitement but more research still needed. Focus on catalysts, Dec.2009; 2009 (12); 2.
[107] Deng X, Li Y, Fei X. Microalgae: a promising feedstock for biodiesel. Afr J Microbiol Res 3(13):1008–14.
[108] The draft National Algal Biofuels Technology Roadmap (https://e-center.doe.gov/iips/faopor.nsf/UNID/79E3ABCACC9AC14A852575CA00799D99/$file/AlgalBiofuels_Roadmap_7.pdf), U.S. Department of energy; 2009.
[109] Thurmond Will. Algae 2020: advanced biofuel markets and commercialization outlook, 1st ed.; 2009. 460 pp.
[110] Thurmond Will. Five drop in fuels: the next generation. Biofuels International; March 2009.
[111] Thurmond Will. Five key strategies for algae biofuels commercialization. Biofuels International; June 2009.
[112] Thurmond Will, Algae. Advanced biofuel insight. Biofuels International; February 2010.